

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
SUBSTRATES FOR SYNTHESIS OF TRANSCRIPTION PRODUCTS
CORRESPONDING TO TARGET SEQUENCES

Inventor(s): Gary A. Dahl/Jerome J. Jendrisak/Elena K. Davydova/
Lucia B. Rothman-Denes/Svetlana Y. Gerdes

Application No.:
Docket Number: 310307.00003

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Bacteriophage N4 vRNAP promoters

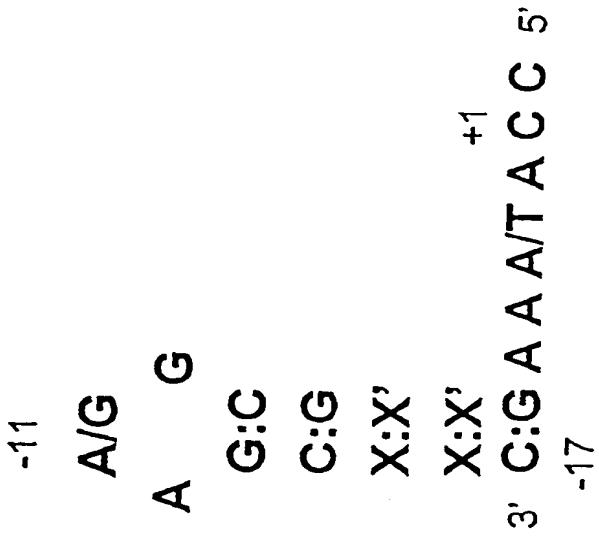


FIG. 1

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N4 vRNAP and generation of mini-vRNAP

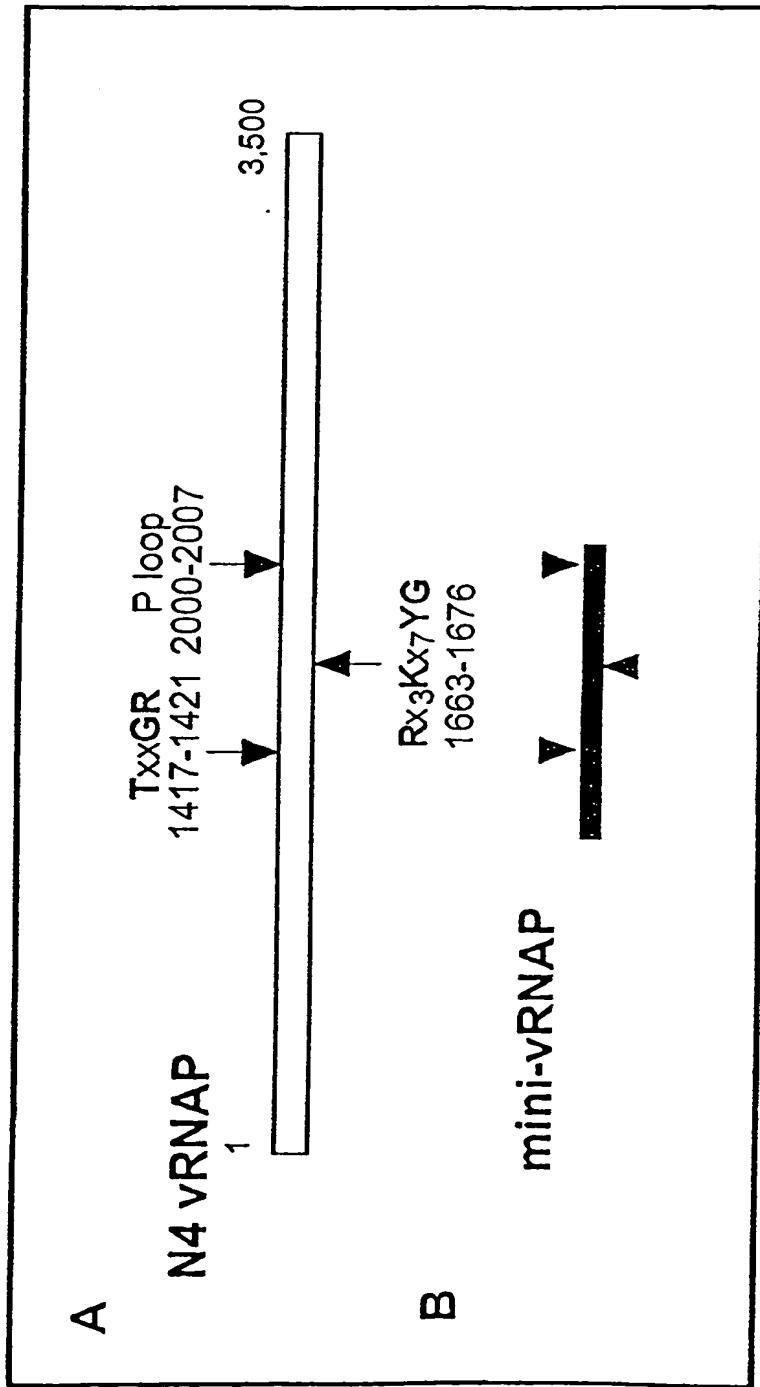


FIG. 2

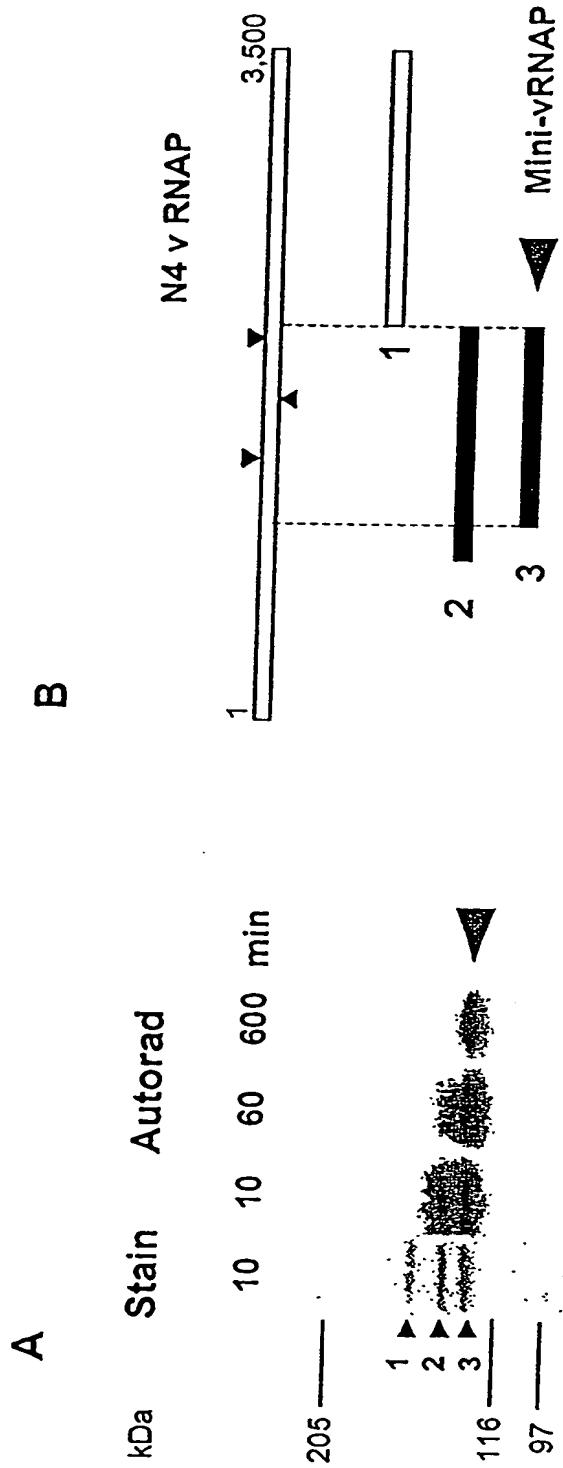
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Identification of the minimal active domain of N4 vRNAP by proteolytic cleavage.



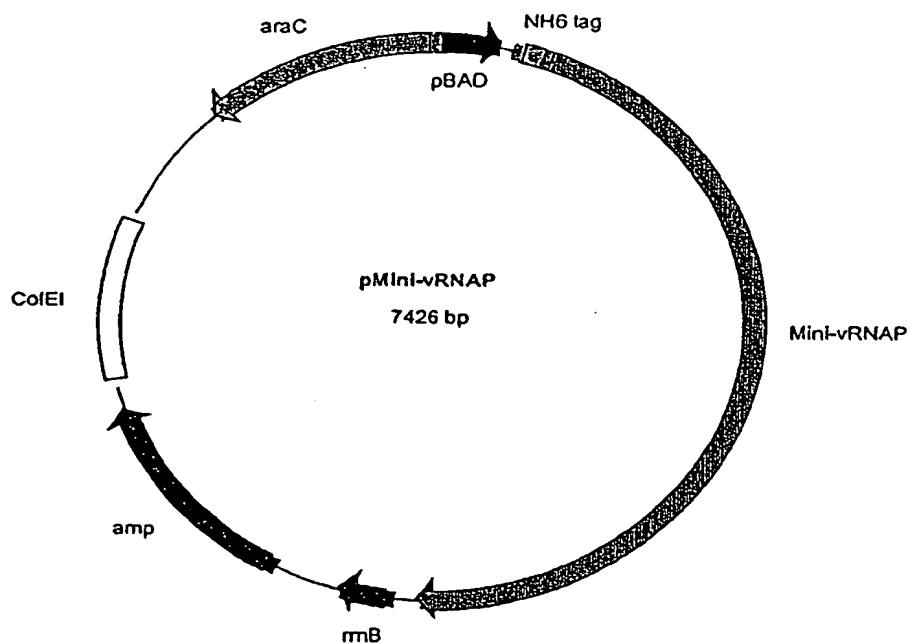
3
FIG.

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Plasmid name: pMini-vRNAP
Plasmid size: 7426 bp
Constructed by: K. M. Kazmierczak
Construction date: 2/2000
Comments: Insert cloned into Invitrogen
pBAD B expression plasmid

FIG 4

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Purification of cloned vRNAP and mini-vRNAP

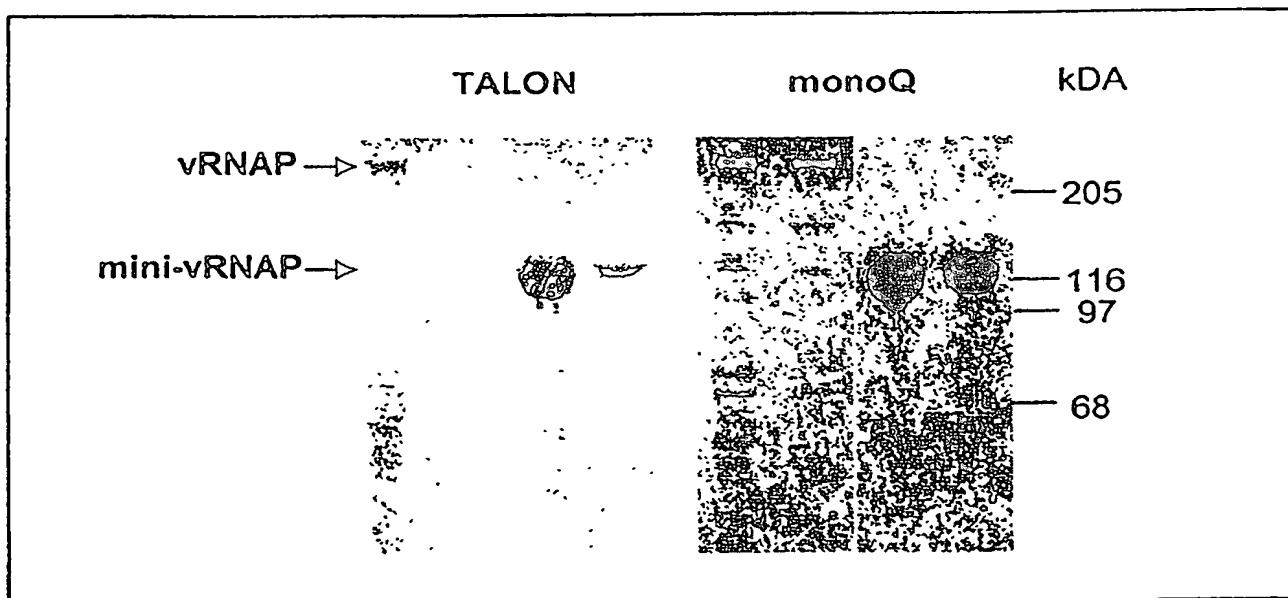


FIG 5

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**Activation of N4 vRNAP transcription by *Eco* SSB
at different ssDNA concentrations**

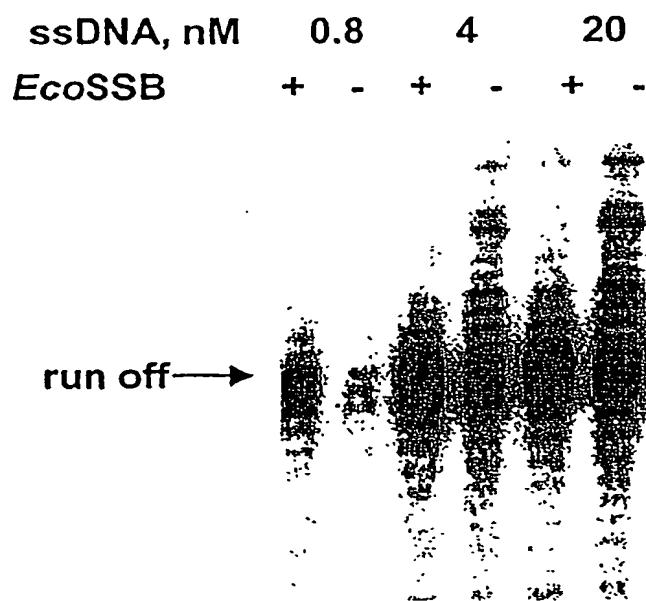


FIG 6

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Effect of *Eco* SSB on ssDNA template recycling

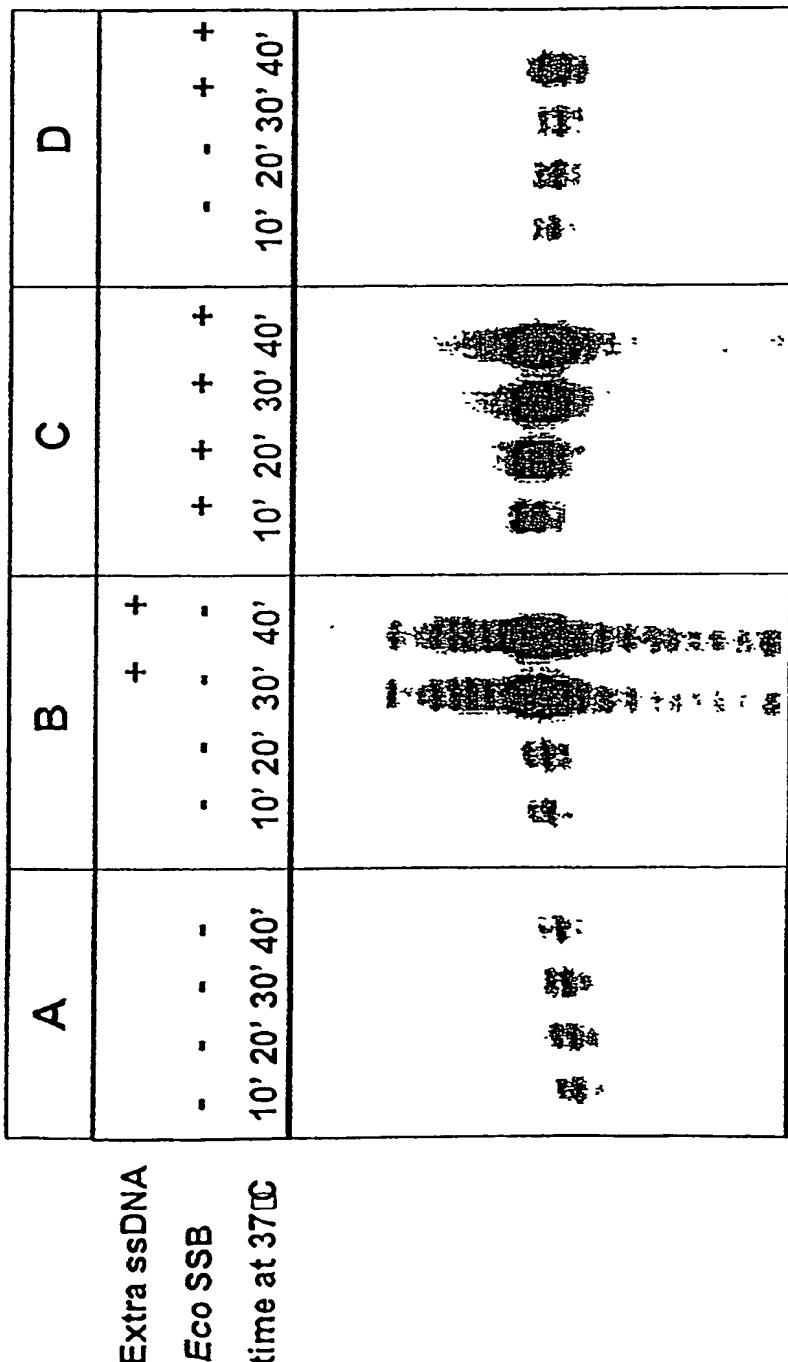


FIG. 7

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**Effect of *Eco* SSB on the state of template DNA
and product RNA in vRNAP transcription**

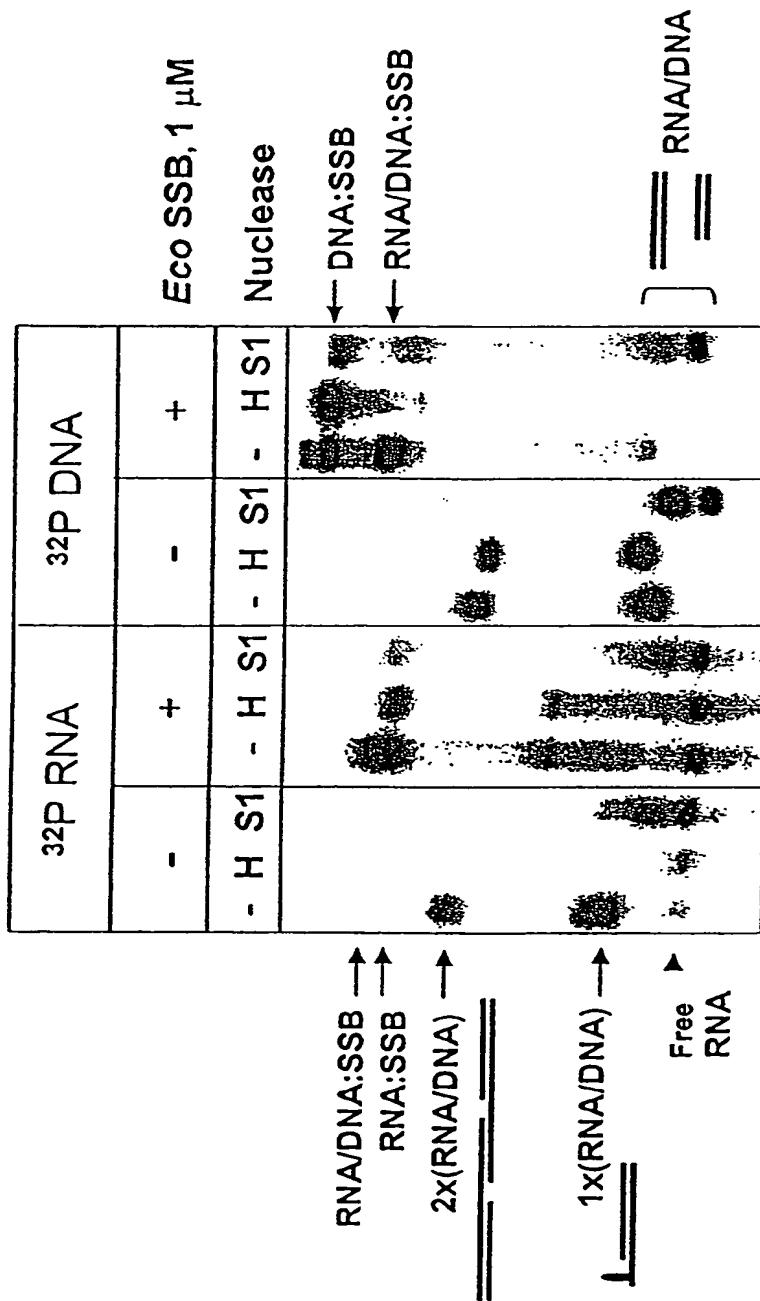


FIG. 8

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION SUBSTRATES FOR SYNTHESIS OF TRANSCRIPTION PRODUCTS CORRESPONDING TO TARGET SEQUENCES

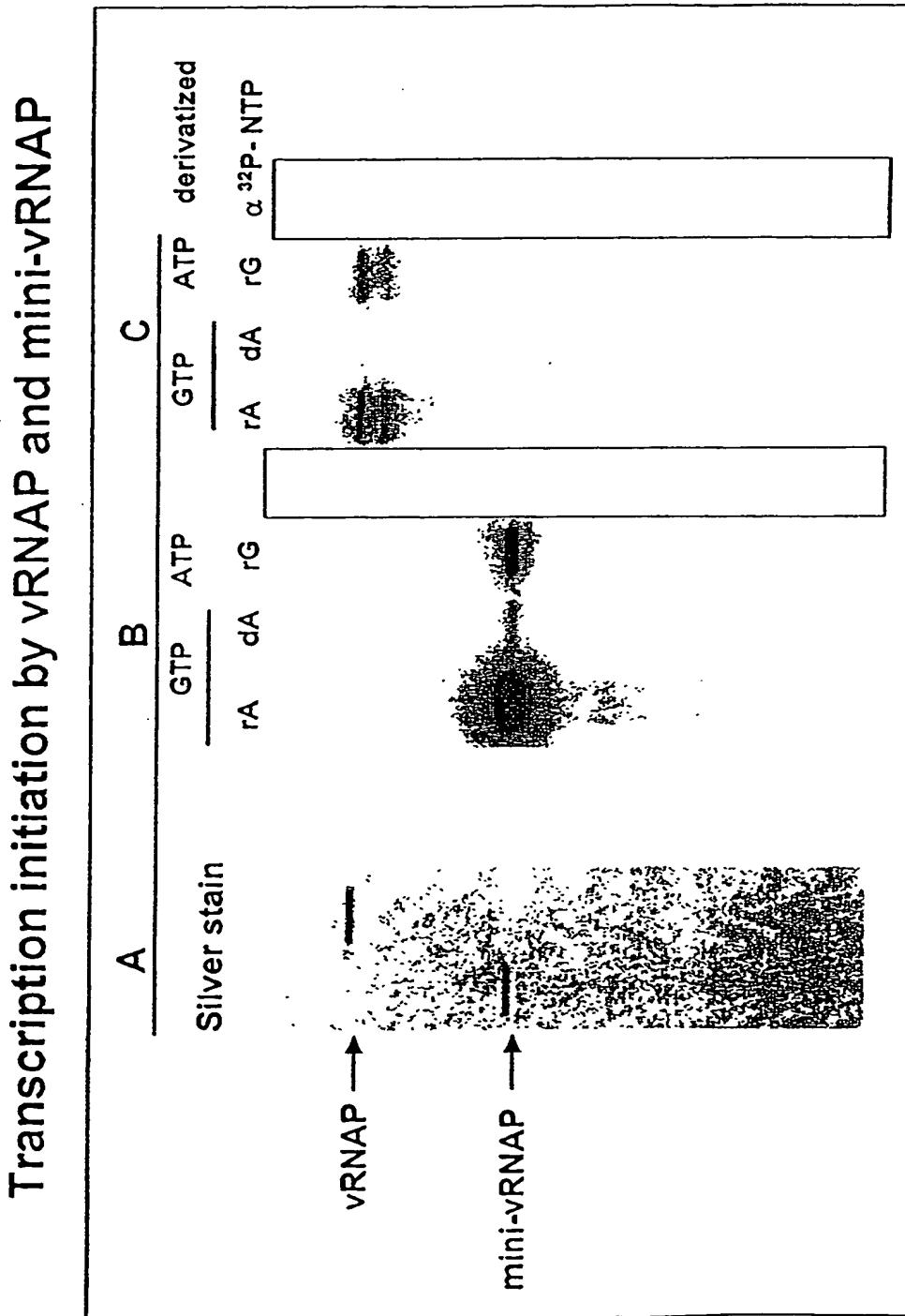
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Effect of *Eco* SSB on transcription of vRNAP and mini-vRNAP

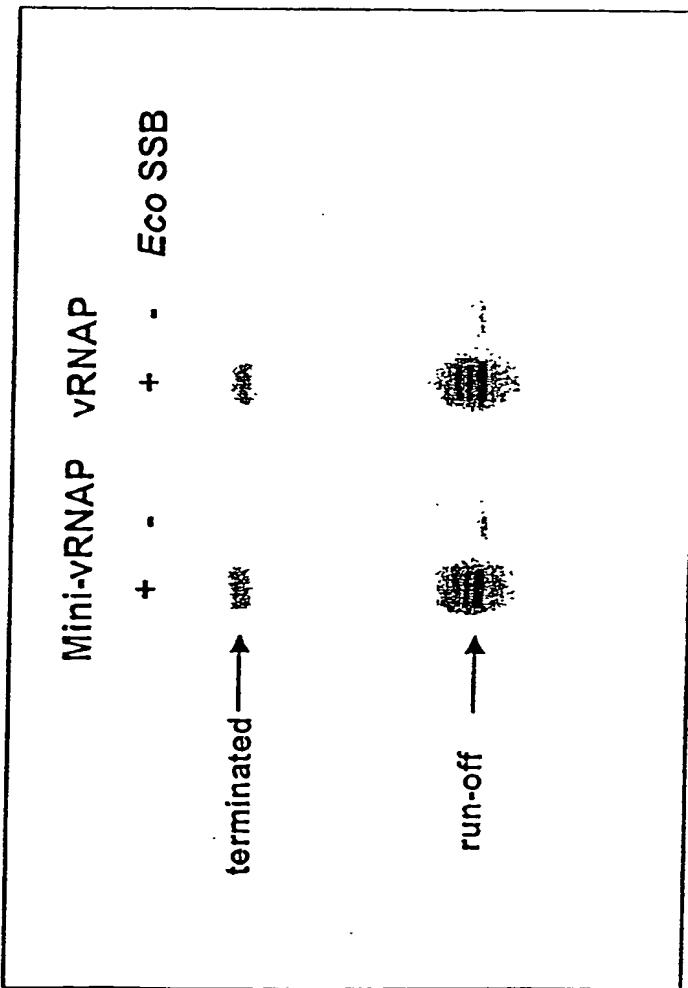


FIG. 10

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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Determination of mini-vRNAP promoter contacts

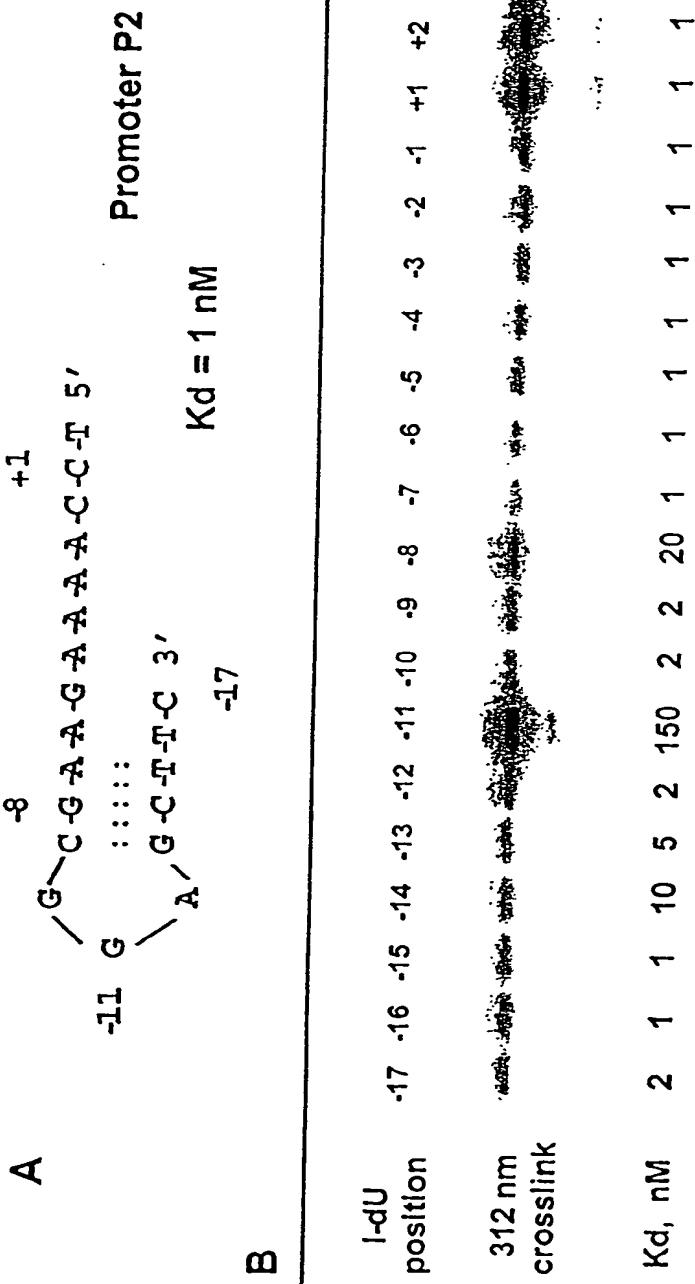


FIG. 11

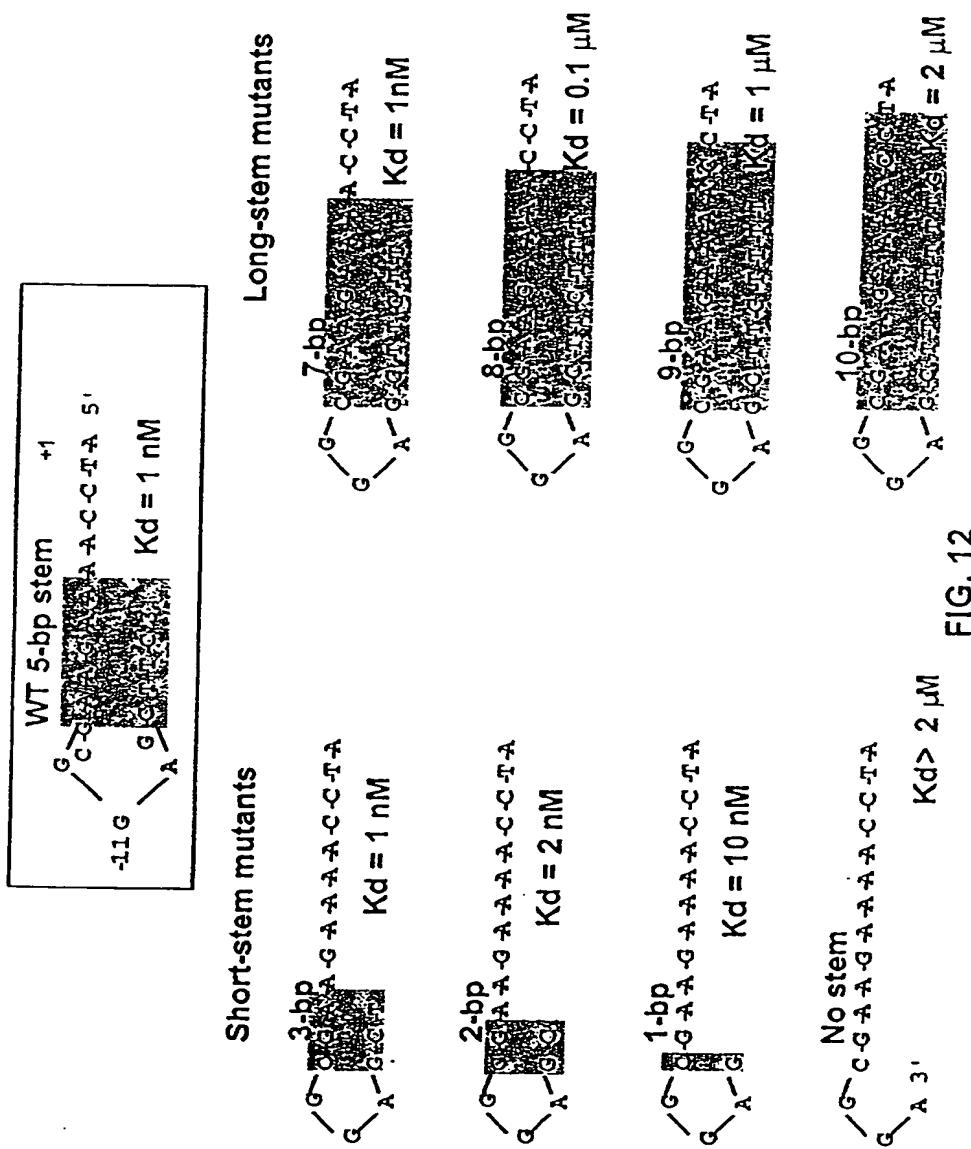
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Binding affinities of stem-length promoter mutants



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Identification of the transcription start site by catalytic autolabeling

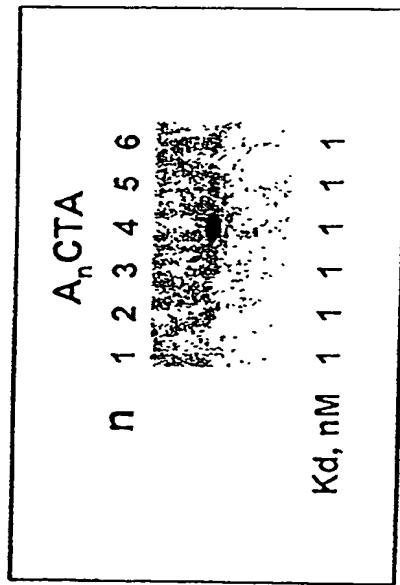
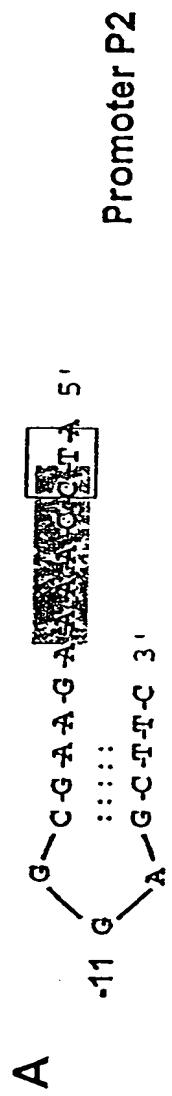


FIG. 13

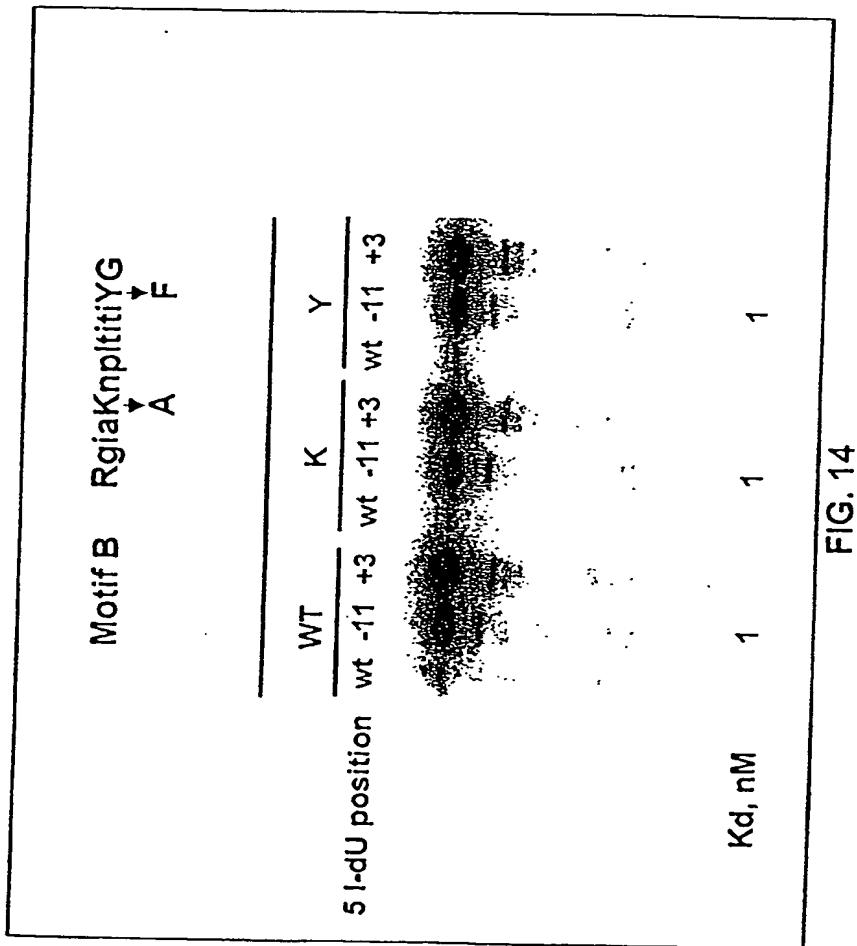
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UV crosslinking of mutant mini-vRNAPases
to promoter oligonucleotides



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Run-off transcription by mutant mini-vRNAPases

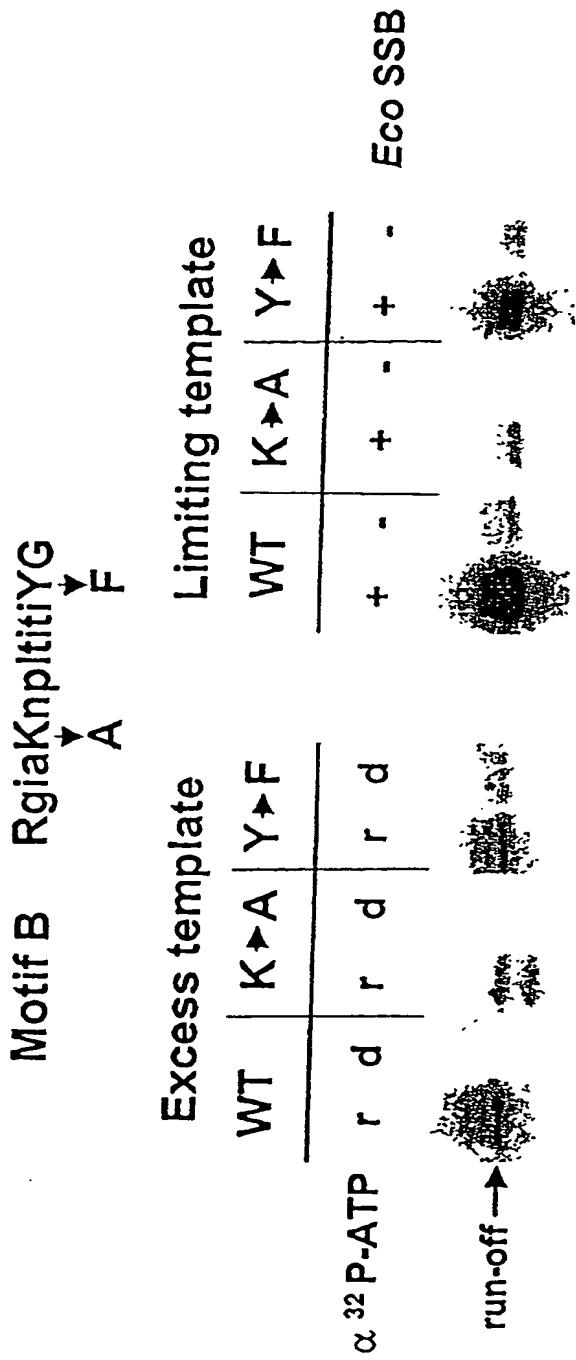


FIG. 15

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Mutant mini-vRNAPases transcription initiation

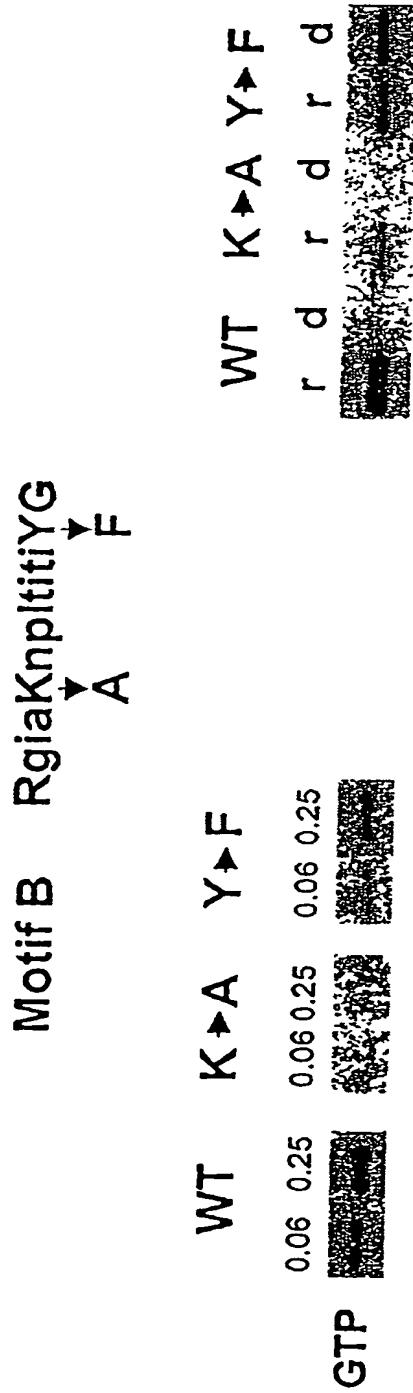


FIG. 16

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Detection of *in vivo* activities of N4 vRNAP and mini-vRNAP

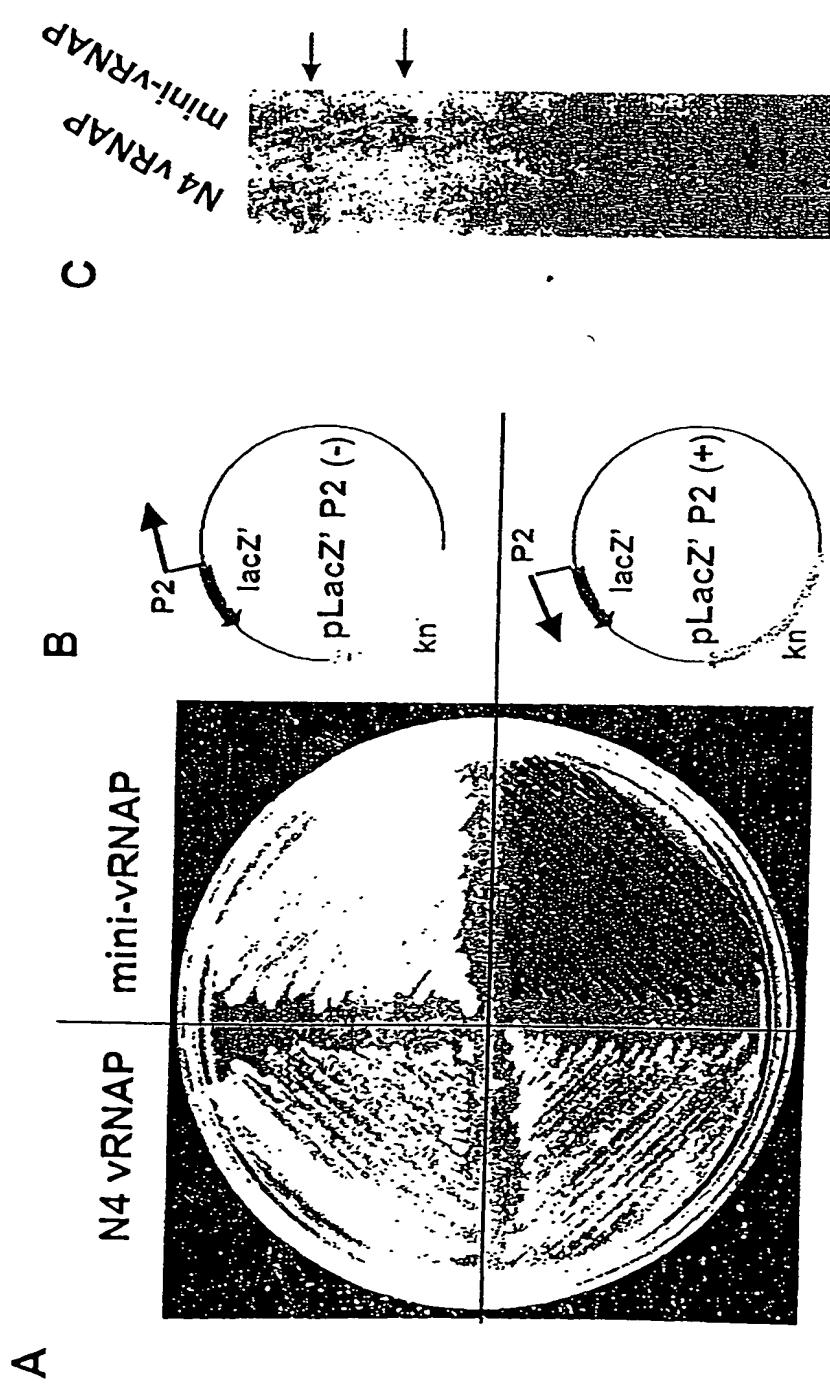


FIG. 17

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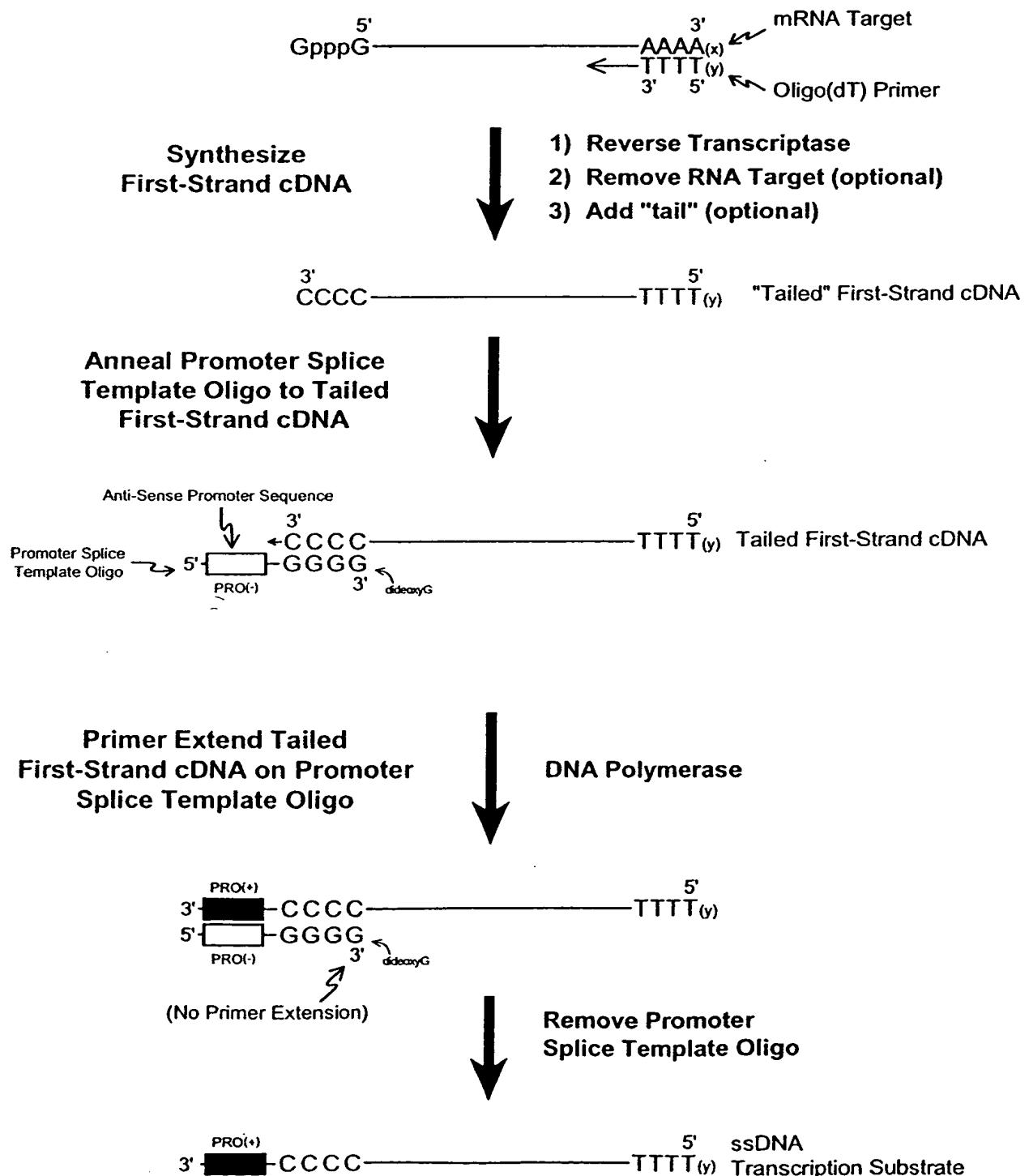


FIG 18

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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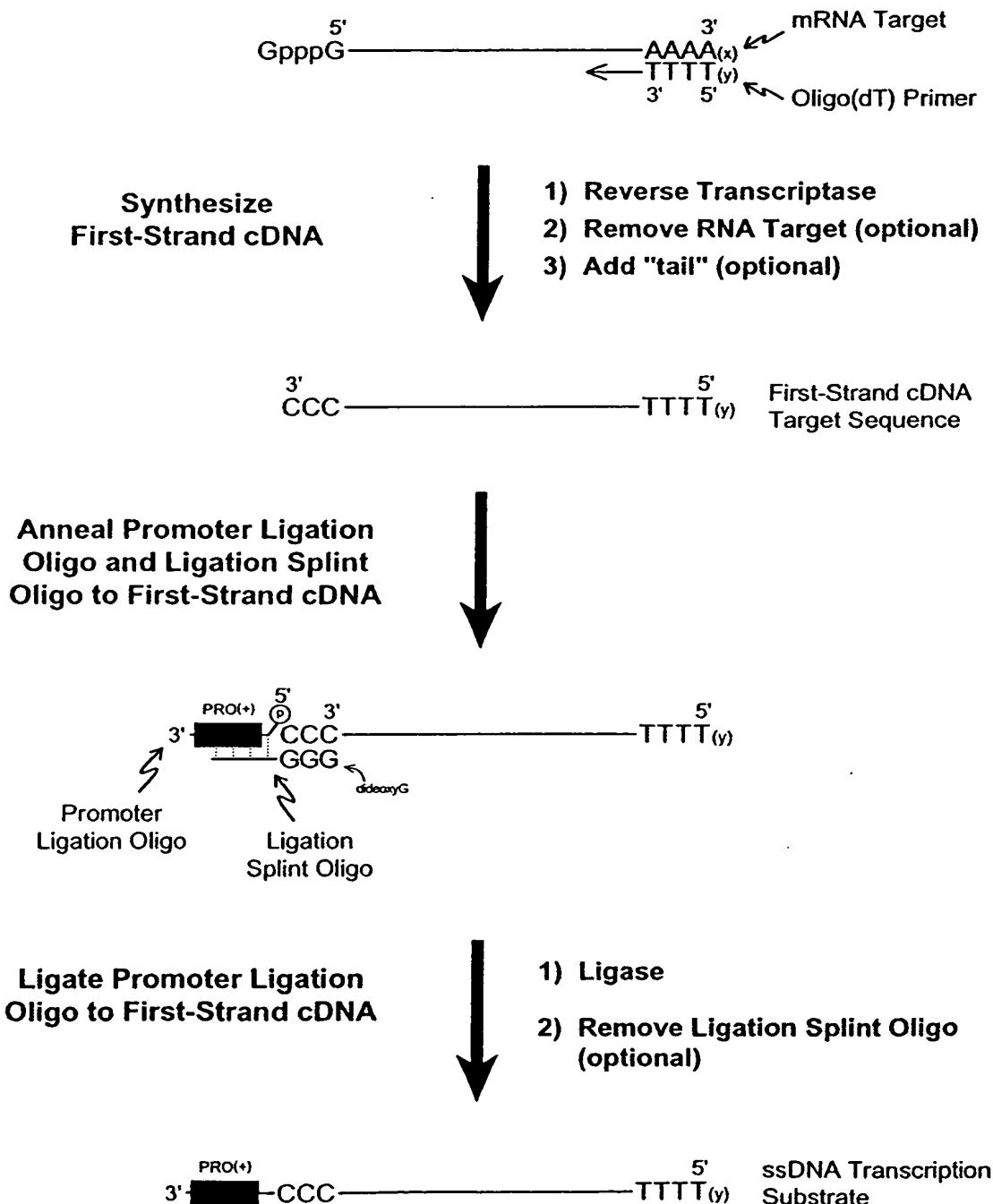


FIG 19

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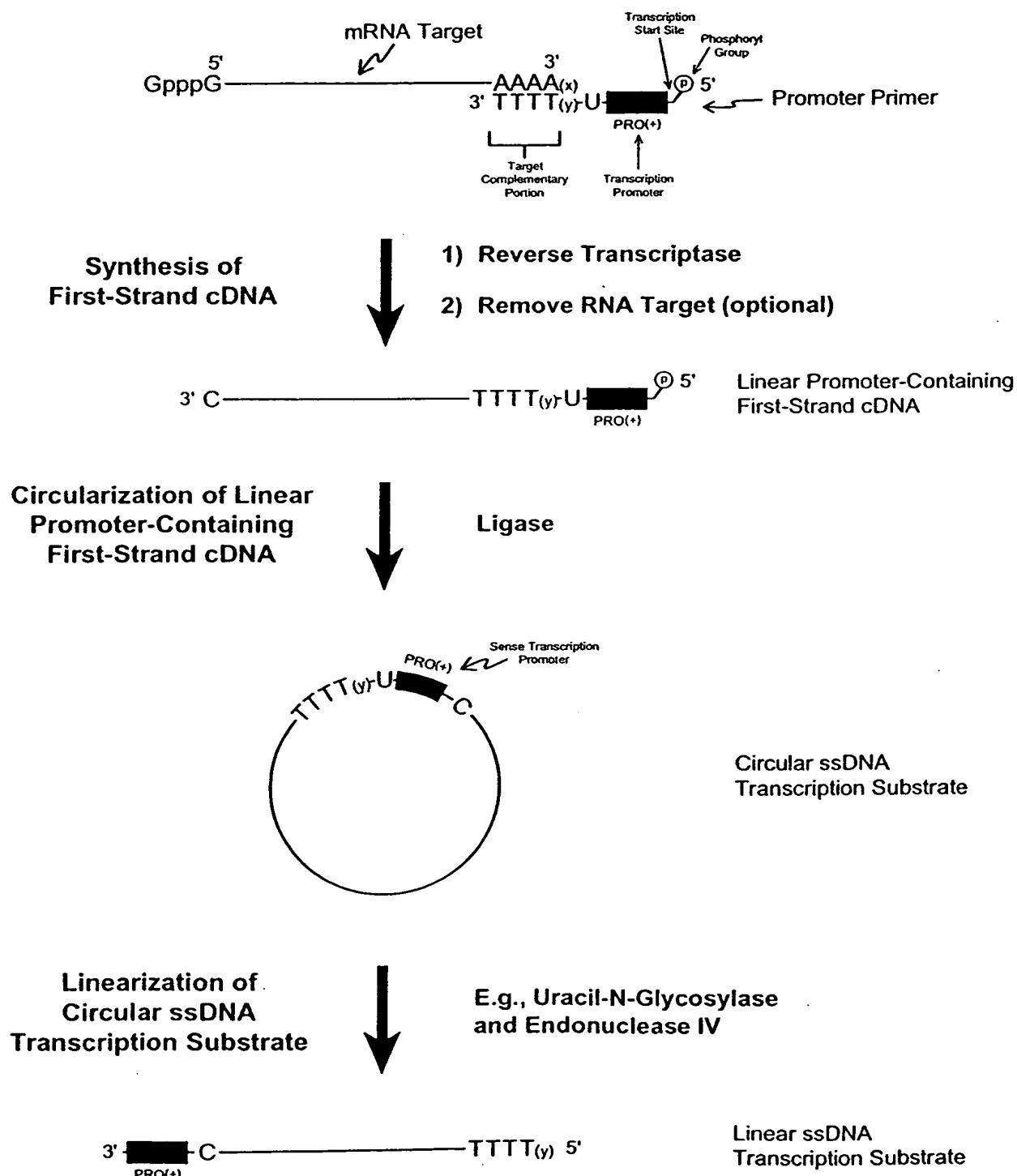


FIG 20

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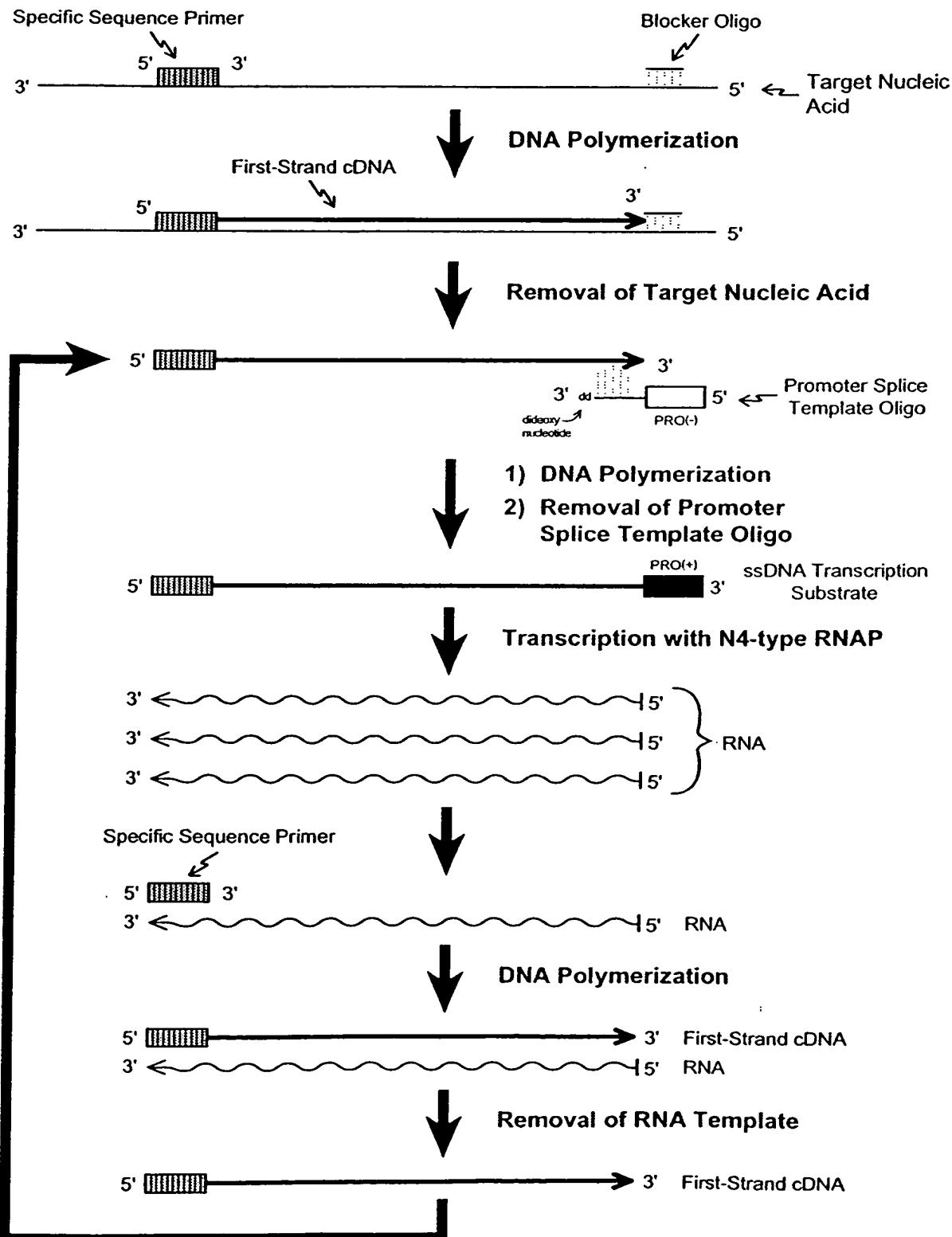


FIG 21

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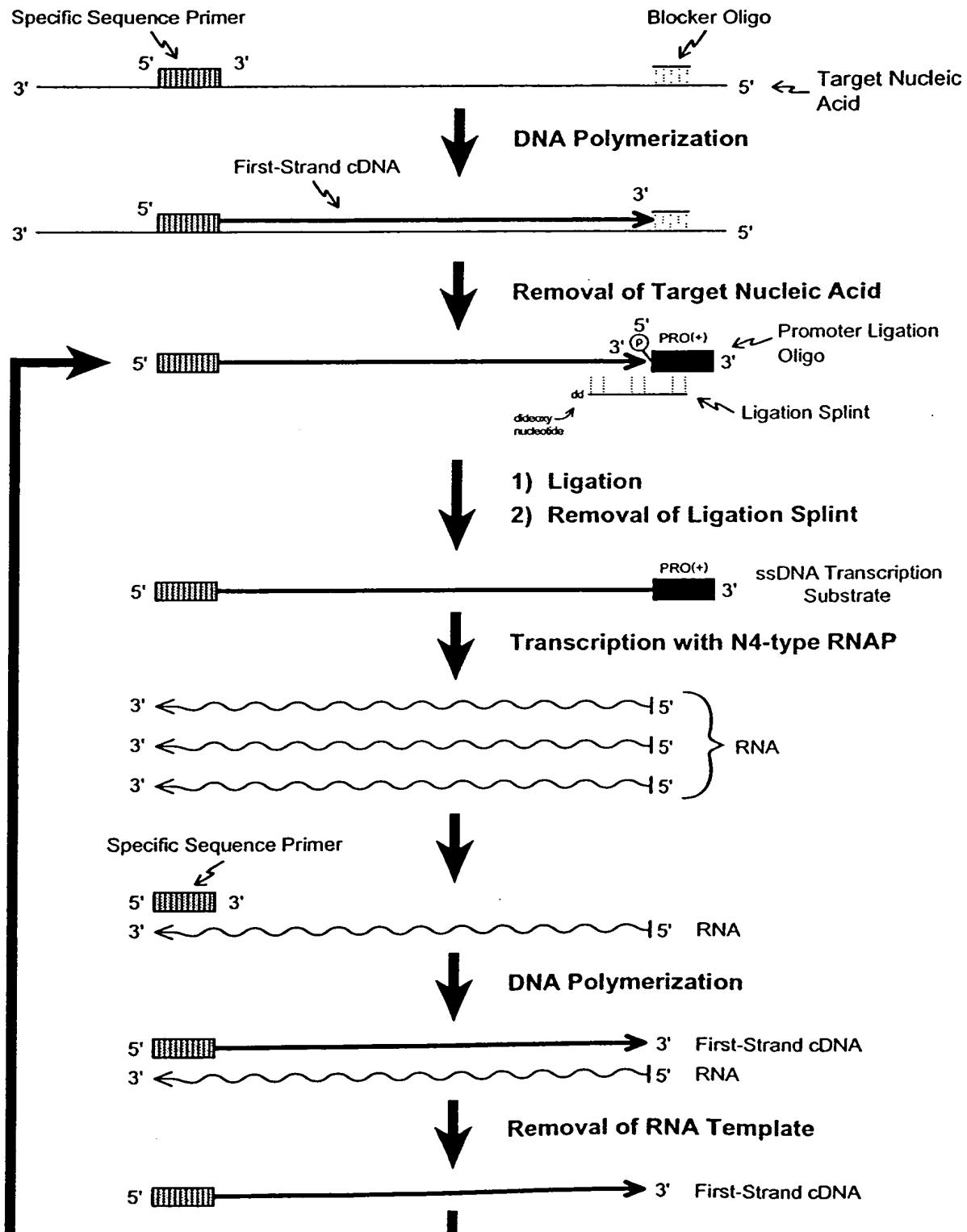


FIG 22

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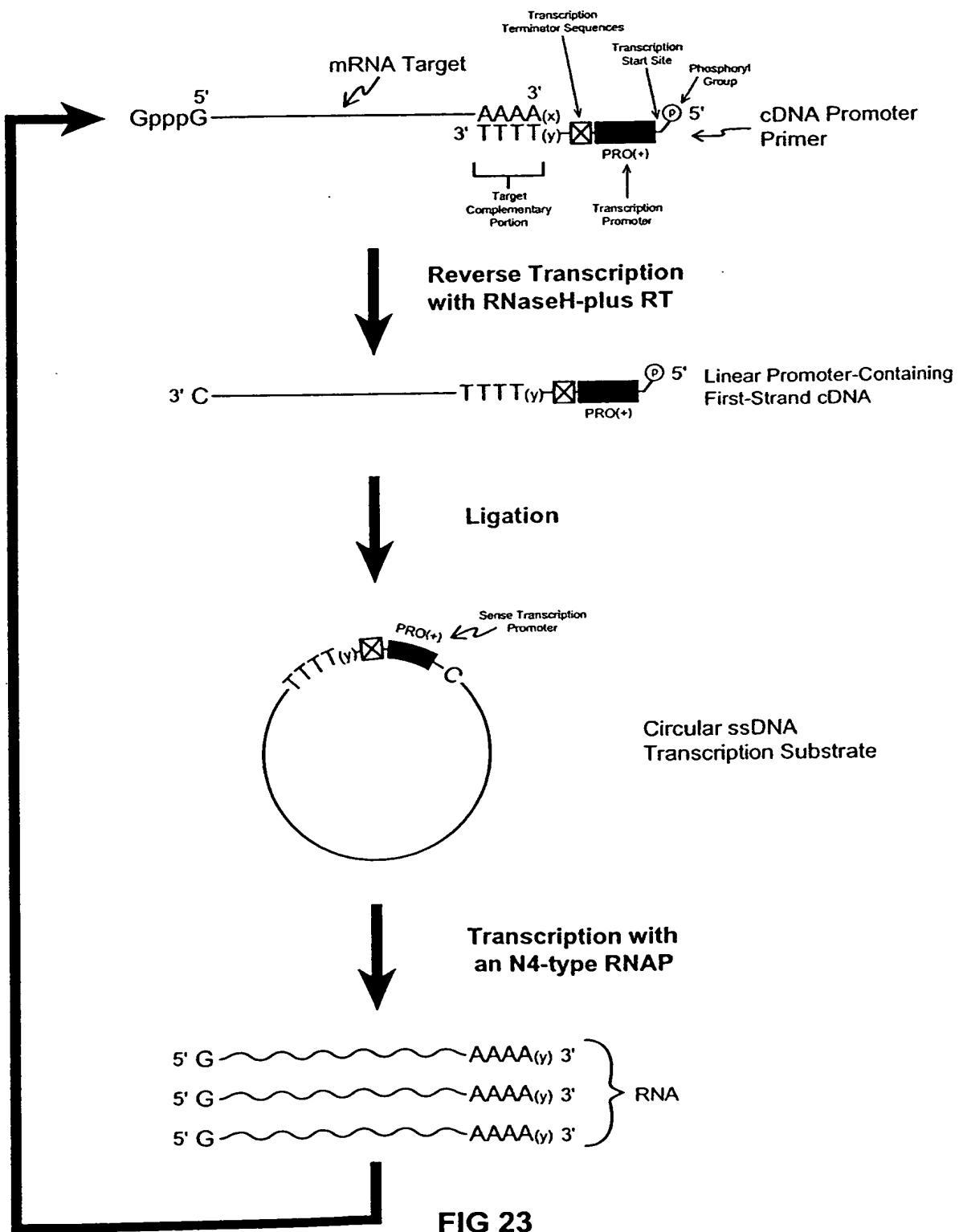


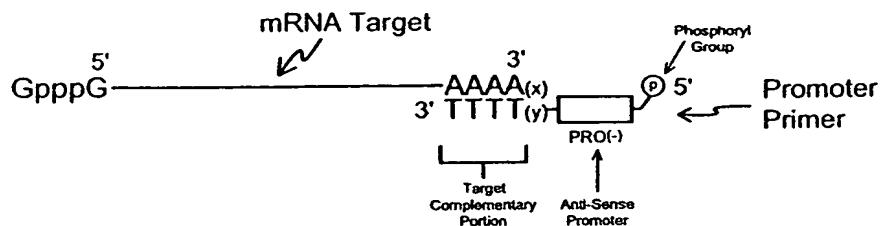
FIG 23

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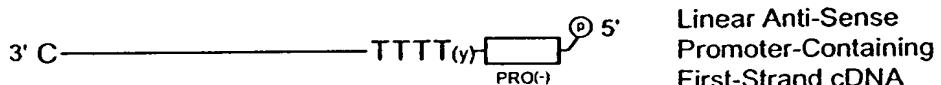
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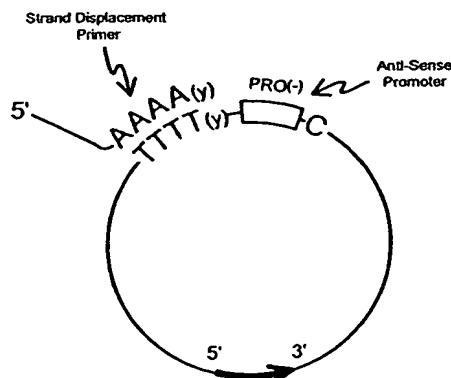
Synthesis of
First-Strand cDNA

1) Reverse Transcriptase
2) Remove Target Nucleic Acid



Circularization of
Linear Anti-Sense Promoter-Containing First-Strand cDNA

Ligase



Circular Anti-Sense Promoter-Containing First-Strand cDNA

Rolling Circle
Replication

Strand-Displacing DNA Polymerase

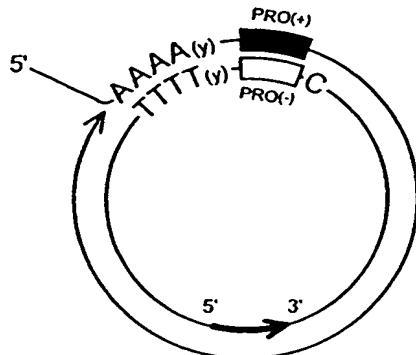
FIG 24 (continued on next page)

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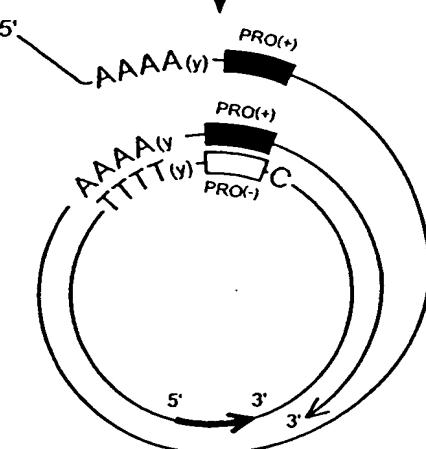
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Rolling Circle Replication
(continued)

Strand-Displacing DNA Polymerase



In Vitro Transcription on
Second-Strand ssDNA
Transcription Substrate

N4 mini-vRNAP and
Single-Strand Binding Protein

